RESEARCH ARTICLE

Variation in ion accumulation as a measure of salt tolerance in seedling and callus of *Stylosanthes guianensis*

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ABSTRACT: Seedlings of *Stylosanthes guianensis* CIAT 184 (Stylo 184) were germinated under salt-stress conditions (0-170.9 mM NaCl), and germination and ion content (Cl^-, Na^+, Ca^{2+}, Mg^{2+} and K^+) were measured after seven days. The salt treatment had no effect on the germination percentage, but the seedling showed reduced growth and displayed variation in ion uptake, thus accumulating Na^+ and Cl^- in the roots. Callus produced from seedlings selected as salt sensitive (T1) or salt tolerant (T2, T3, T4 and T5) was examined for growth and ion content under the influence of NaCl (0-256.4 mM NaCl) after 15 days. The sensitive clone (T1) contained the lowest Na^+ and Cl^- content with the highest percentage of K^+ contributing to osmolality, suggesting it possesses an ion regulation mechanism that is typical of glycophytes; i.e., osmotic potential was adjusted by accumulating K^+. The other clones (T2, T3, T4 and T5) expressed different strategies (osmotic adjustment) to cope with salt stress. T5 showed the highest mean dry weight on salt medium and displayed more effective ion regulation by maintaining low Na^+:K^+ and Na^+:Ca^{2+} ratios. T2 expressed high Na^+ and Cl^- with the highest percentage of Na^+ contribution to osmolality and water content (succulence). T3 and T4 had lower growth rates but similar ion regulation in relation to T5. Results indicated that the ion content can be used to discriminate salt sensitive and salt tolerant clones of Stylo 184.

KEYWORDS: forage legume, ion exclusion, osmolality, osmotic adjustment.

INTRODUCTION

Soil salinisation is one of the most essential problems in agricultural production throughout the world (Munns 2002, Flowers 2004). Approaches to overcoming this problem have been both physical and biological. Physical approaches generally concentrate on controlling water regimes, and hence the transportation of salts in irrigation and in soil profiles (Mostafazadeh-Fard et al. 2007). Biological approaches concentrate on the mechanisms that lead to salt tolerance and on the subsequent selection of these characteristics in a variety of plants. Munns (2002) suggested several approaches to recognize salt tolerance in crop plants, including the regulation of ion uptake. Examining these physiological aspects of salt tolerance has led to a range of promising results for the increased production under saline conditions. The production of salt-tolerant crops, however, does not necessarily address the cause of the problem, and without improved irrigation methods and alleviation of soil salinisation caused in dryland salinity (by the alteration of groundwater levels), the problem will be aggravated.

Legumes are a prime example of plants that have several functions because they contribute with soil nitrogen content and provide products such as crops or fodder. This refers especially to deep rooted perennials, as they are able to alleviate problems with rising water tables. The use of legumes to address saline soil problems has been widely recommended, however, legumes are generally sensitive to salt, and this fact has limited their application (Betteridge and Jones 2001, Ashraf and Iram 2005, Al Sherif 2009, Saha et al. 2010).

Legumes are generally classified as glycophytes. Salt-tolerant glycophytes are able to deal with salt stress by relying on a range of mechanisms including osmotic adjustment, specific ion exclusion/transport, compartmentalization, or a combination of them (Munns 2002, Tejera et al. 2006, Zakharin and Panichkin 2009). Within the glycophytes, however, different species display a range in salt tolerance. With legumes, ion exclusion has been identified as a major tolerance mechanism (Luo et al. 2005). Nevertheless, overproduction and accumulation of organic osmolytes (i.e., proline and reducing sugars) have also been reported (Sidari et al. 2008, Arulbalachandran et al. 2009, Amirjani 2010). Some legumes, such as chickpea, are able to accumulate Na^+ and K^+ in their roots, which decrease the root osmotic potential (Tejara et al. 2006). Other salt-tolerant glycophytes such as *Aster tripolium* (Ueda et al. 2003) and *Trachyspermum ammi* have also shown osmotic adjustment with the accumulation of Na^+ and Cl^- (Ashraf and Orooj 2006).

It appears that K^+ uptake under salt stress may be particularly important in-salt tolerant glycophytes. It is essential to maintain the cytoplasmic reactions and it is presumed to be acting as a nonorganic solute, as the levels of accumulation are insufficient to lead to osmotic adjustment (Yokoi et al. 2002). Also, K^+ and Mg^{2+} have been reported to play an important role in enzyme activation (Barker and Pilbeam 2007). The role of Mg^{2+} is unclear under salt stress, since responses have been variable *in vitro*. For instance, it increases in rice callus (Ahmad et al. 2009), decreases in soybean callus (Liu and Staden 2001), and is unaffected in callus of *Sonneratia alba* J. Smith (Yasumoto et al. 1999).

Stylosanthes species are important forage legumes that originate from the tropical Americas. *S. guianensis* CIAT 184 (Stylo 184) is widely grown in sub-tropical and tropical regions including the Philippines, Australia, Indonesia, Malaysia, Vietnam, Laos, and Thailand. It has exceptional forage value and high protein content (approximately 14–18% foliage) and is well adapted to a range of soil types from sand to light clay, but is sensitive to saline and sodic soils (pH>8.5) (Phengsavanh and Ledin 2003, Homma et al. 2008).

Some of the best investigations of the traits in relation to salt tolerance are those associated with the ion contents of plants grown under saline conditions (Flowers 2004) and in the past decade, *in vitro* selection for salt tolerant lines has been reported as being sucssessful (Hassan et al. 2004, Woodward and Bennett 2005, Bekheet et al. 2006, Kashyap and Sharma 2006). In this study, we hypothesized that ion regulation in plant tissue contributes to salt tolerance in Stylo 184, and we aimed to determine whether ion regulation in seedlings or callus could be used to select salt tolerant individuals.

MATERIAL AND METHODS

Germination and growth: Seeds of Stylo 184 were obtained from the Animal Nutrition Division, Department of Livestock, Thailand. Seeds were soaked in warm water at 80° C for 1–2 min in order to break dormancy and then were immersed in 1% NaOCl for 30 min followed by five rinses in sterile distilled water. Surface-sterilized seeds were placed in Petri dishes (20 seeds per dish) and exposed to 2 mL of 0, 85.5, 128.2 and 170.9 mM NaCl for seven days. A completely randomized design (CRD) was used with five replicates. Germination percentage, shoot height, root length, and ion content (Ca^{2+}, Mg^{2+}, Na^+ and K^+) in shoots and roots were determined after the treatments (see details below).

Growth and ion content in callus under salt stress: Based on our preliminary work and following salt-tolerant selection in potatoes (Jefferies 1996) and tomatoes (Foolad 1996), seeds of Stylo 184 were treated with 0-512.8 mM NaCl. It was found that the 341.9 mM NaCl concentration was the highest at which seeds were able to germinate. In addition, germination was not significantly different from 0 to 170.9 mM. Salt-tolerant clones were therefore selected by germinating 60 g (approximately 37,500 of the sampling seeds from the same seed lot as the aforementioned one) in a sterile 341.9 mM NaCl solution. Every 100 mg surface-sterilised seeds were germinated in a Petri dish containing a 10 mL NaCl solution. Each germinated seed was evaluated for its salt tolerance (T2-T5). One sensitive clone (T1) was derived from a seed which did not germinate with 170.9 mM NaCl, but germination was observed when salt stress was removed.

All of the five selected clones of stylo 184 (T1, T2, T3, T4 and T5) were then induced to form callus for four weeks by culturing on MS medium (Murashige and Skoog 1962), supplemented with 0.01 mg L^{-1} 6-alpha-naphthaleneacetic acid (NAA) and 1 mg L^{-1} benzyladenine (BA) at 25°C under a 16-hour photoperiod (Veraplakorn et al. 2012). The callus of all clones was then proliferated on the same medium by subculture twice

every four weeks. Their salt-tolerant capacity was examined by exposing the callus to the same medium, but with 0, 85.5, 170.9 and 256.4 mM NaCl. The experiment was set up using a factorial CRD and the relative growth rate (RGR = 100 × final dry weight stress/final dry weight control) (Yacoubi et al. 2010), and ion content was determined after 15 days.

Nutrient analysis and osmolality: At the end of the experiment, the seedlings and callus were dried up at 50°C for two days in a hot air oven. Samples were taken and ground into fine powder and 0.1 g ground samples were digested in 10 mL of HNO_3: $HClO_4$ acid mixture (2:1, v/v) and kept at room temperature for one day. Samples were then heated at 100–150°C until being fully digested. Deionized water was added to adjust the final volume of 50 mL and filtered with a 0.2 μm membrane filter (Ruiz et al. 1997). These samples were then analysed as to Ca^{2+}, Mg^{2+}, Na^+ and K^+ content using atomic absorption and flame emission spectrophotometer (Perkin Elmer, USA).

Cl^- was determined by grinding 0.1 g of each sample, mixing with 0.1 g CaO and 1 mL deionized water, and then combusting at 500°C for 3 h. The ash was dissolved with 50 mL deionized water, and afterwards a 0.5 mL subsample was removed and mixed with 4.5 mL deionized water. The solution was added to 0.5 mL mercury thiocyanate (0.075%) and 1.0 mL ferric nitrate nonahydrate (4.04 %). Cl^- content was measured from the colour of the ferric thiocyanate complex with absorbance at 460 nm and compared with a standard Cl^- curve (Adriano and Doner 1982).

For osmolality analysis of the cell sap, fresh callus was frozen at -80°C. After thawing, the callus was placed into a 1 mL syringe and the syringe plunger was pressed to express cell sap. Molality was determined by a Wescor model 5100C vapour pressure osmometer (New Jersey, USA). This sap osmolality value was multiplied by -2.48 to reach osmotic potential. The molal concentrations of Na^+ and K^+ were converted to osmolality by multiplying by 1.84 (Bell and O'Leary 2003).

Statistical analysis: A completely randomized design (CRD) was performed for germination percentage. The factorial experiment under CRD was carried out for other parameters, depending on whether the experiment involved seedlings (NaCl concentrations and organs) or clones (NaCl concentrations and clones). Equal variances were tested with Levene's test. When significant differences were found due to treatment, Tukey's B multiple range test was applied. Differences were considered significant when $p \leq 0.05$. All analyses were performed using the software PASW Statistics 18.

RESULTS

Germination, growth and ion content in seedling: The control group germinated in sterilized water illustrated normal growth with the highest shoot and root lengths. Salt treatments of 0 to 170.9 mM NaCl had no significant effects on germination percentage (Figure 1A). However, NaCl reduced seedling growth. Shoot and root lengths of seedlings germinated in 85.5 mM NaCl were significantly lower than those of the control group, while seeds exposed to 128.2 mM and 170.9 mM NaCl showed even greater reduction, but no significant differences from one other (Figure 1B).

There was significant variation among salt treatments in both shoots and roots for Na^+, and Cl^- (Figure 2). As NaCl increased, the mean Na^+ and Cl^- contents increased significantly, while K^+, $Mg^{2+}$and Ca^{2+} contents remained constant. In addition, Na^+, Cl^-, K^+ and Mg^{2+} concentrations were higher in roots than in shoots (Figure 2). Na^+:K^+ ratios increased significantly at 85.5 mM NaCl for both shoots and roots (Figure 3A). The Na^+:Ca^{2+} ratios, however, did not

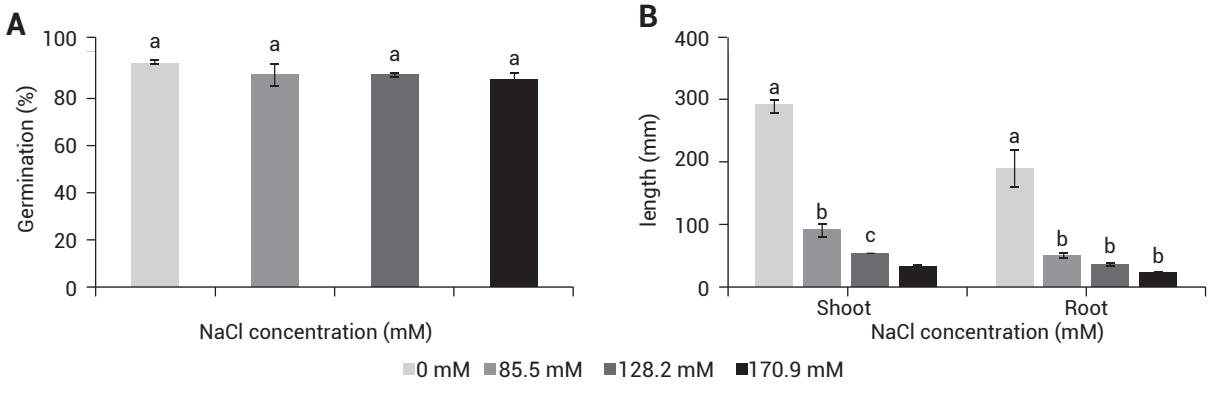

Figure 1. Percent germination of stylo 184 seeds (A), and growth of 7-d-old seedlings (B) after treatment with 0–170.9 mM NaCl. Error bars indicate standard error; n=5; different letters above bars indicate significant difference ($p \leq 0.05$).

change in shoots, but significantly increased in roots (Figure 3B). A reduction in water content was observed only in shoots at 170.9 mM NaCl. In roots, however, water content was stable with increasing NaCl (Figure 4).

Growth and ion content in callus: For each of the measured parameters, the two-way ANOVA indicated there was a significant difference caused both by clone and treatment with the exception of Mg^{2+} content. In addition, there was a two-way interaction between the clones and treatment, indicating that not all clones responded the same way. The callus RGR of the five clones under the influence of NaCl (0–256.4 mM) varied both by clone and concentration with growth generally being reduced in increasing salt concentrations (Figure 5). Whereas T1, T2 and T4 showed reductions in RGR from 85.5 mM NaCl, T3 displayed them only from 170.9 mM NaCl. On the other hand, T5 only had significant reduction at 256.4 mM NaCl (Figure 5).

Callus water content was also significantly reduced as NaCl concentration increased (Figure 6). However, while there were differences between clones, it did not reflect those seen in callus growth. T2 was able to maintain its water content at 85.5 mM NaCl with a significant reduction at 170.9 mM NaCl (compared to control), but there was no further reduction at 256.4 mM NaCl. All the other clones had significant reduction in water content at the lowest NaCl concentration, of 85.5 mM.

The potential osmotic of all clones decreased significantly with the enhancement of NaCl concentration (Figure 7A). In addition, as NaCl increased, the contribution of Na^+ and K^+ to osmolality varied among clones. The highest contribution of Na^+ was found in T2, while the lowest one was found in T1. In clones T2, T3 and T5 there was an increasing contribution of Na^+, but T1 and T4 remained stable from 85.5 to 256.4 mM NaCl. Also, the contribution of K^+ to osmolality in T2, T3, T4 and T5 decreased significantly, but it was stable in all levels of NaCl for T1. T1 had the highest contribution of K^+ to osmolality (36.7%) and K^+ was also the major contributor (59.3%) in the medium without NaCl (Figure 7B).

The ion content of the callus depended on the clone and level of NaCl in the medium. Na^+ and Cl^- were significantly higher for each clone with increasing NaCl application (Figures 8A, B). Based on the two-way ANOVA, T1 increased the least with growing NaCl and T2 increased the most for both Na^+ and Cl^-. The other three clones had intermediate responses. For K^+, there was a significant difference among the clones and the response to increasing NaCl varied again. In T1 and T2, there was no significant difference and also no change in K^+ over the four NaCl concentrations. However, the concentrations were twice as high in T1 compared to T3, T4 and T5 (Figure 8C). In addition, T5 was able to stabilize its K^+

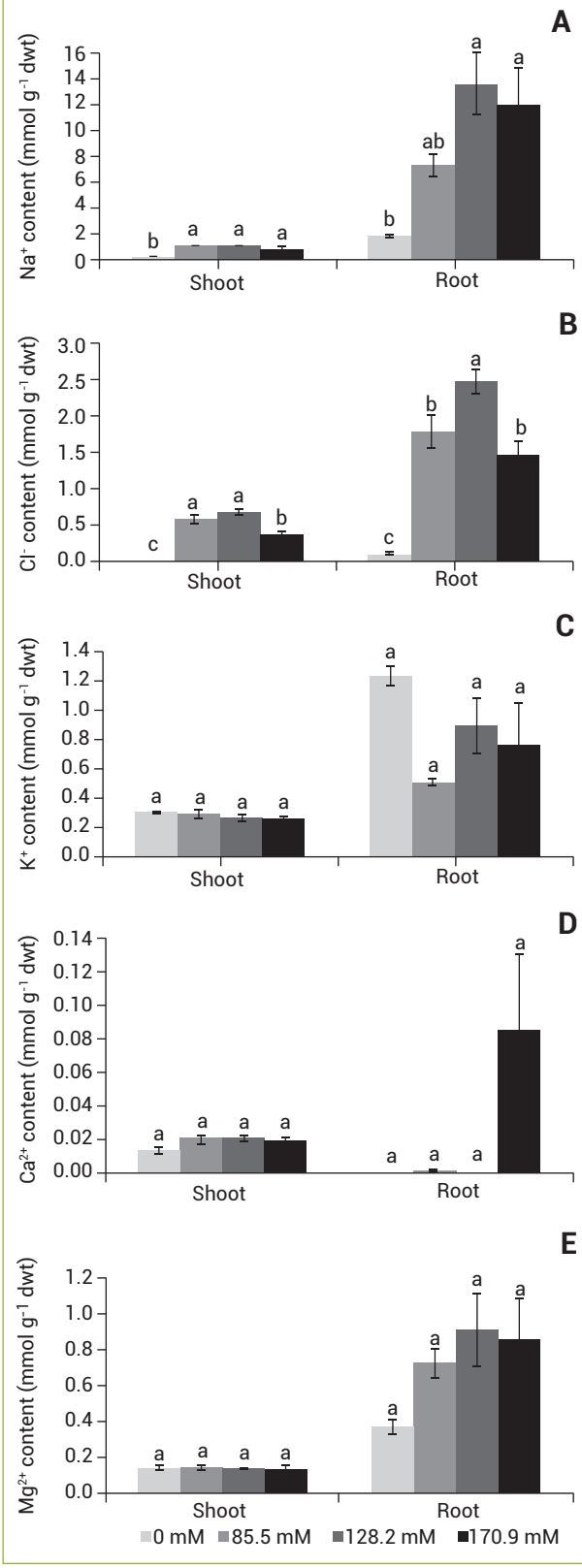

Figure 2. Ion content in 7-d-old seedlings of stylo 184 after treatment with 0–170.9 mM NaCl. Na^+ (A), Cl^- (B), K^+ (C), Ca^{2+} (D), and Mg^{2+} (E). Error bars indicate standard error; n=5; different letters above bars for each organ indicate significant difference ($p \leq 0.05$).

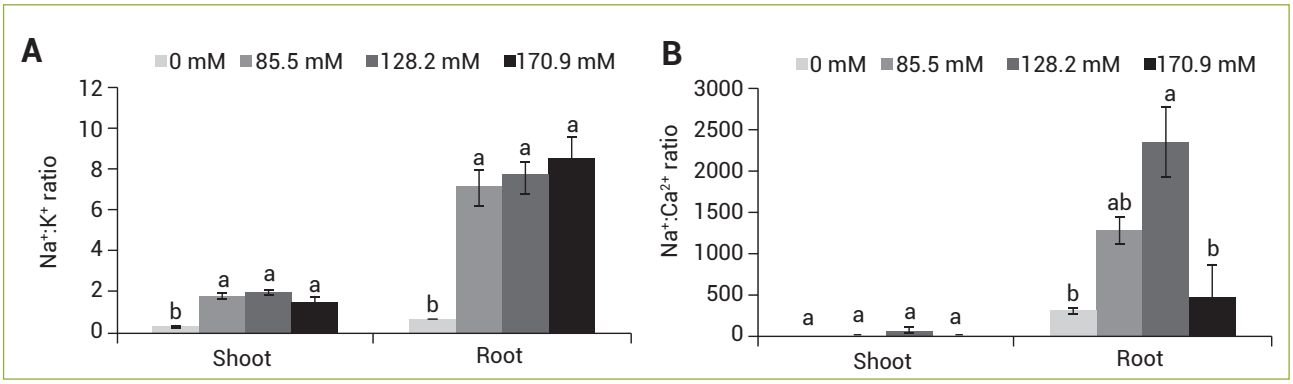

Figure 3. Ion ratios in 7-day-old seedlings of stylo 184 after treatment with 0–170.9 mM NaCl. Na^+: K^+ (A), and Na^+: Ca^{2+} (B). Error bars indicate standard error; n=3; different letters above bars for each organ indicate significant difference ($p \leq 0.05$).

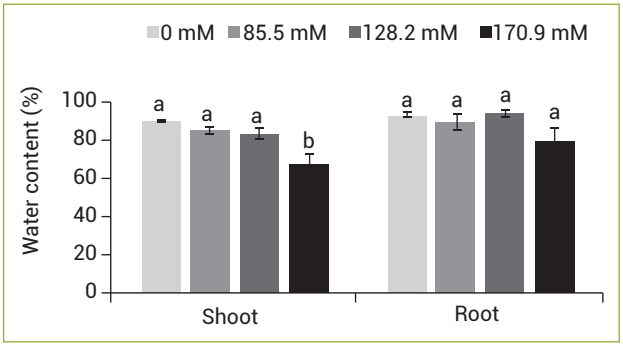

Figure 4. Water content in 7-day-old seedlings of stylo 184 after treatment with 0–170.9 mM NaCl. Error bars indicate standard error; n=5; different letters above bars for each organ indicate significant difference ($p \leq 0.05$).

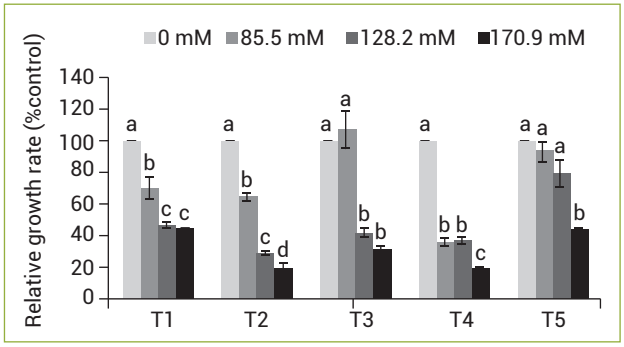

Figure 5. Growth of callus from stylo 184 selected clones after treatment with 0–170.9 mM NaCl for 15 days. Error bars indicate standard error; n=10; different letters above bars for each clone indicate significant difference ($p \leq 0.05$). T1, T2, T3, T4 and T5 are selected clones of Stylo 184.

levels at all NaCl concentrations, while T3 and T4 decreased K^+ with increasing NaCl (Figure 8C).

Even though T1 and T2 had the same trend in retaining K^+ content, T2 was also able to maintain Ca^{2+} and Mg^{2+}. However, in T1, Ca^{2+} was reduced in the salt medium while Mg^{2+} increased mainly at 170.9 mM NaCl (Figures 8D, E). T3, T4 and T5 showed strong reduction in Ca^{2+} content already at 85.5 mM NaCl, whereas only T3 showed decrease in Mg^{2+} content, which was observed from 170.9 mM NaCl (Figures 8D, E).

The variable changes in the ion content of clones led to significant differences in the Na^+:K^+ ratios and the Na^+:Ca^{2+} ratios. Based on the two-way interaction, as a result of high K^+ and low Na^+, T1 had the lowest Na^+:K^+ ratio. T2, however, showed the opposite effect with a significantly higher Na^+:K^+ ratio than all the other clones (Figure 9A). The other clones had intermediate responses. T5 had no significant differences in the Na^+:K^+ ratio from 85.5 to 170.9 mM NaCl, while the other clones increased. T3 had the highest Na^+:Ca^{2+} ratio. Only T2 maintained a stable Na^+:Ca^{2+} ratio when exposed to NaCl (Figure 9B).

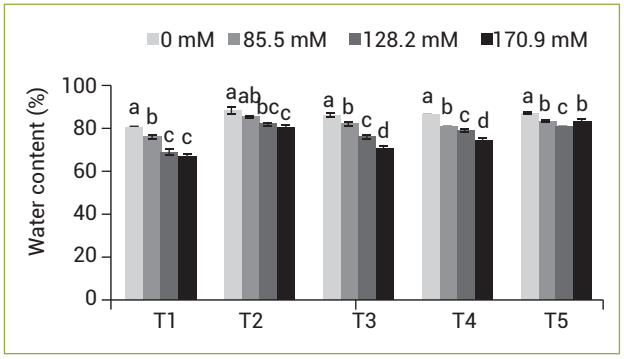

Figure 6. Water content of callus from stylo 184 selected clones after treatment with 0–170.9 mM NaCl for 15 days. Error bars indicate standard error; n=10; different letters above bars for each clone indicate significant difference ($p \leq 0.05$). T1, T2, T3, T4 and T5 are selected clones of Stylo 184.

DISCUSSION

Germination, growth, and ion content in seedling: NaCl (up to 170.9 mM) reduced seedling growth, but had no effect on percentage germination, and this result was similar

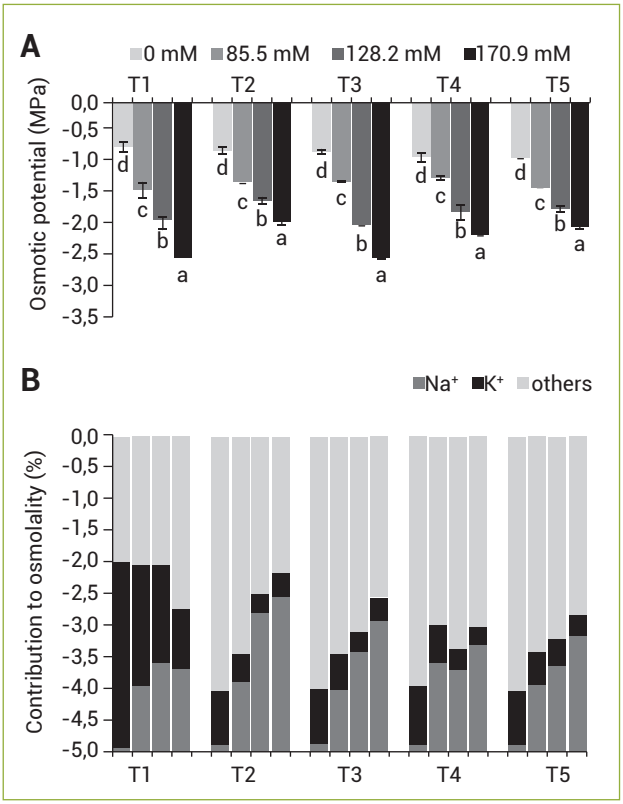

Figure 7. Cell sap determination of callus from stylo 184 selected clones after treatment with 0-170.9 mM NaCl for 15 days. Osmotic potential (A) and contribution to osmolality by Na^+ and K^+ (B). Error bars indicate standard error; n = 3; different letters above bars for each clone indicate significant difference ($p \leq 0.05$). T1, T2, T3, T4 and T5 are selected clones of Stylo 184.

to that reported for a range of other species (Chartzoulakis and Klapaki 2000, Shannon et al. 2000, Bayuelo-Jimenez et al. 2002). For other legumes, NaCl of approximately 341.9 mM or more reduced germination and growth, e.g. soybean (Hosseini et al. 2002) and cowpea (Patel et al. 2010). Similarly, our preliminary study showed that concentrations of NaCl at 256.4 mM had an effect on germination (reduced to 22%), and the highest NaCl concentration in which the seeds were able to germinate was 290.6 mM NaCl (unpublished result). The significant reduction in water content achieved at 170.9 mM NaCl can be attributed to osmotic effects caused by the reduced water uptake by the seedlings (Shannon et al. 2000, Bayuelo-Jimenez et al. 2002, Jamil et al. 2007).

The Na^+ and Cl^- contents in the shoots and roots of Stylo 184 seedlings increased when treated with NaCl. The result, again, reflects what has been frequently reported, that is, Na^+ and Cl^- contents are higher with increasing salinity (e.g. Chartzoulakis and Klapaki 2000, Hosseini et al. 2002, Patel et al. 2010). However, it appeared that the Cl^- content in shoots and roots was lower in the treatment with 170.9 mM NaCl than in that with 128.2 mM NaCl, and this may be related to water content. Such a relationship between Cl^- and water content was

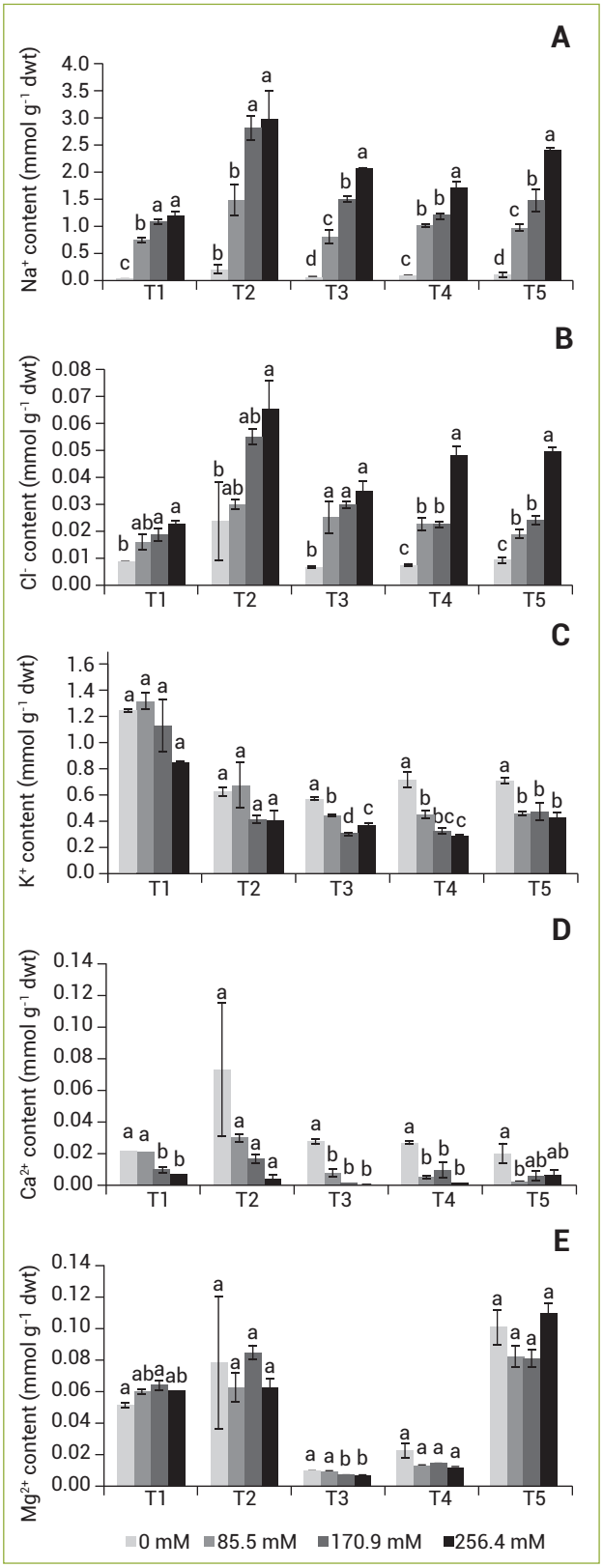

Figure 8. Ion content of callus from stylo 184 selected clones after treatment with 0-170.9 mM NaCl for 15 days. Na^+ (A), Cl^- (B), K^+ (C), Ca^{2+} (D), and (E) Mg^{2+}. Error bars indicate standard error; n=3; different letters above bars for each clone indicate significant difference ($p \leq 0.05$). T1, T2, T3, T4 and T5 are selected clones of Stylo 184.

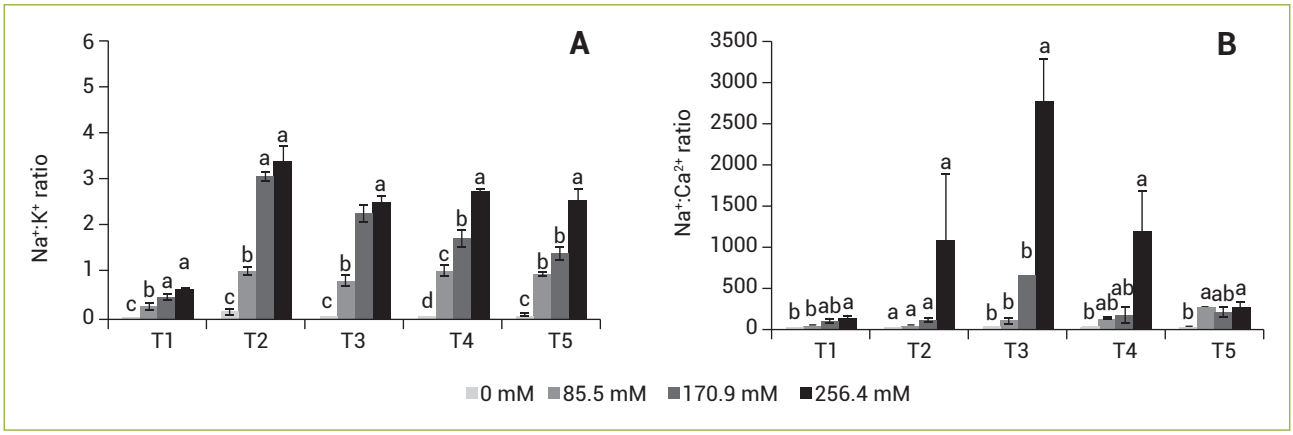

Figure 9. Ion ratios of callus from stylo 184 selected clones after treatment with 0–170.9 mM NaCl for 15 days. Na^+: K^+ (A), and Na^+: Ca^{2+} (B). Error bars indicate standard error; n=3; different letters above bars for each clone indicate significant difference ($p \leq 0.05$). T1, T2, T3, T4 and T5 are selected clones of Stylo 184.

reported in both sensitive and tolerant clones of *Citrus*, in which total chloride was highly correlated with total water content (Moya et al. 2003). In addition, stylo 184 was able to prevent the accumulation of toxic ions in the shoots. Lupins (*Lupinus luteus* and *L. angustifolius*) also reduce the translocation of both Na^+ and Cl^- to the leaves and stems (Van Steveninck et al. 1982, Teakle et al. 2007), while clover (*Trifolium repens*; Rogers et al. 1997, Wang et al. 2010) and wild soybean (*Glycine soja;* Luo et al. 2005) control the translocation of Cl^- or Na^+ to the growing shoots, respectively. This suggests that stylo 184 displayed an exclusion mechanism by accumulating more Na^+ and Cl^- in roots than in shoots.

As a result of salt stress, plants usually face potassium deficiency, as K^+ uptake is limited by high concentrations of Na^+ and xylem translocation is restricted (Patel et al. 2010, Tavakkoli et al. 2010). In Stylo 184, K^+ content was significantly higher in roots than in shoots, and the total content was 5-7 times lower than the Na^+ content. This is similar to other legumes, such as cowpea *(Vigna anguiculata)*, faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), soybean (*Glycine max* L.), and common bean (*Phaseolus vulgaris* L.) (Cordovilla et al. 1995, Patel et al. 2010). The germination of stylo 184 was stable, possibly due to the preferential K^+ transport and the reduction of K^+ translocation to shoots. However, this fact, and perhaps the accumulation of toxic ions, apparently led to reduced seedling growth.

At higher levels of salt (85.5–170.9 mM), stylo 184 was able to maintain both the K^+ content and the K^+:Na^+ ratio in both shoots and roots. Plants are able to cope with salt stress by maintaining a high cytoplasmic K^+:Na^+ ratio (Blumwald 2000, Marcum et al. 2007). As reported in soybean, germination at a higher tissue of Na^+ concentration in stylo 184 was associated with higher K^+ and Ca^{2+} concentrations in the embryo axis (Hosseini et al. 2002). Farhoudi and Sharifzadeh (2006) reported that canola seeds primed with NaCl subsequently showed high K^+ and Ca^{2+} accumulation, including a well-balanced Na^+:Ca^{2+} ratio, apparently preventing toxic effects and nutrient deficiency. This type of regulation may have played an important role in osmotic adjustment for stylo 184 seeds, leading to germination in 170.9 mM NaCl.

Growth and ion content in callus of selected clones:

Examination of callus growth clearly showed different growth rates and an apparent difference in salt tolerance of the five selected clones, as indicated by relative growth rate, ion regulation and osmotic adjustment. It is possible that a combination of these factors will lead to the level of salt tolerance of individual genotypes (Flowers 2004, Zakharin and Panichkin 2009, Zhou and Yu 2009).

In the present results, callus of T1 (salt-sensitive) displayed the highest K^+ content and consequently the lowest Na^+:K^+ ratio. It also contained the highest percentage of K^+, contributing to osmolality, with low osmotic potential, resulting in higher mean dry weight of T1 than T2 and T4. This may have been achieved by T1, which successfully adjusted the osmotic potential by accumulating K^+ in the cells. This consumes less energy than the production and accumulation of organic osmolytes, and it would be beneficial to its salt adaptation (Zhou and Yu 2009), i.e. a typical glycophytic response.

T2 was able to stabilize K^+, Ca^{2+} and Mg^{2+}, but it showed higher Na^+ and Cl^- content with increasing NaCl. Possibly, this clone may have used the Na^+ and/or Cl^- to adjust its osmotic potential. The high osmotic potential with high Na^+ and Cl^- content, as well as the highest mean water content among all the clones, may be considered as succulence and this physiological mechanism is used to survive under salinity stress (Khan et al. 1999). Great succulence (particularly in

the whole plant) can be a key characteristic to evaluate the potential of germplasm in selection and breeding programs for improving salt tolerance (Ottow et al. 2005, Lacerda et al. 2006, Gulzar and Khan 2006). This was different from the response obtained from other clones, which had lower water content. The growth of T2 was also reduced at higher salt levels in comparison to the other clones selected as "tolerant" (T3, T4 and T5). This may be partly due to the excessive toxic ions in the cytoplasm and for losing high energy through the accumulation of these ions in the vacuole.

T5 had the slowest growth amongst all clones, but it showed what appeared to be the highest tolerance to salt in terms of having the lowest growth reduction when exposed to NaCl. The regulation of K^+, Ca^{2+}, and Mg^{2+} in this clone led to stable Na^+:K^+ and Na^+:Ca^{2+} ratios. Marcum et al. (2007) suggested that the high Na^+:K^+ ratio can disrupt various enzymatic processes in the cytoplasm. Salt-tolerant plants respond to elevated Na^+ concentrations by maintaining low cytosolic Na^+ concentrations with high cytosolic K^+:Na^+ ratios through the extrusion and/or intracellular compartmentalization (Blumwald 2000). For example, callus of *Distichlis spicata* tolerant genotypes had a higher K^+:Na^+ ratio than sensitive genotypes (Marcum et al. 2007). Under the current conditions, T5 appears to have better control of K^+, and hence was able to maintain growth at higher NaCl concentrations.

Five clones were selected according to their germination ability under salt stress conditions as a criterion to indicate tolerance. The mechanism used by non-selected seedlings (seven days old) to overcome the secondary effect of salt stress should be related to the clones selected as tolerant. The results presented in this research indicate that exclusion mechanisms play an important role for stylo 184 seedlings to survive under salt stress conditions. The salt sensitive clone (T1) displayed typical responses of glycophytes, including high accumulation of K^+ and maintenance of low levels of Na^+ and Cl^- (Sairam and Tyagi 2004). This approach eventually leads to the reduced growth or death if the exposure is prolonged or if the salt stress increased (Zakharin and Panichkin 2009). On the other hand, the variation of osmotic adjustment found in the clones selected as tolerant (T2, T3, T4 and T5) revealed salt-resistant glycophyte mechanisms by accumulating, to various degrees, Na^+ and Cl^-, as well as K^+, as osmolytes. These results indicate that higher preferential K^+ accumulation can be used as a criterion to discriminate salt-sensitive clones from clones of stylo 184 selected as being tolerant to salt. Understanding these differences may be useful to increase the salt tolerance of this plant for its inclusion in the restoration of salt affected soil as well as its use as a fodder plant.

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